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# Design of a simple device for accurate measurement of human blood viscosity in oxygenated and deoxygenated conditions

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**Design of a simple device for accurate measurement of human blood viscosity in oxygenated and deoxygenated conditions**

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**Abstract**

Our client, Dr. George Lykotrafitis of the Biomedical and Mechanical Engineering Departments at the University of Connecticut, requested the design of a simple, portable, inexpensive device to measure whole blood viscosity (WBV) under oxygenated and deoxygenated conditions. WBV is affected by diseases such as sickle cell disease (SCD) and cardiovascular disease, conditions that can lead to serious or life-threatening conditions and largely affect individuals in areas with little access to healthcare [2][3][4][5]. While WBV is recognized as a point-of-care measurement for conditions such as SCD and cardiovascular disease, the use of conventional rheometers in areas without healthcare facilities is not practical as testing methods are expensive, time consuming, require large blood samples, and/or are not usable outside of a laboratory setting [5][6]. Here, a microfluidic rheometer is proposed for the measurement of WBV. The device employs capillary pressure to drive the flow of a reference fluid of known viscosity and a blood sample through a microfluidic array. The WBV of the blood sample is calculated using the known channel dimensions, capillary pressure, reference fluid viscosity, and sample fluid velocity. The microfluidic device will be fabricated using a wet chemical etching method and has a projected cost of approximately $1.40 per device if produced on a commercial scale. The proposed device is novel in that it requires a small blood sample, is portable, does not require power, and allows for measurement of WBV over a range of shear rates that are clinically meaningful. It overcomes the obstacles that limit the operation of currently available viscometers outside of a laboratory setting and meets the client specified objectives of portability, simplicity, and cost.

## 1. Introduction

### *1.1 Background*

Our client, Dr. George Lykotrafitis of the Mechanical and Biomedical Engineering Departments at the University of Connecticut, is seeking a novel device to measure whole blood viscosity WBV in a simple, cost-effective manner. Dr. Lykotrafitis directs the Cellular Mechanics laboratory at the University of Connecticut. The laboratory's research includes work involving sickle cell disease SCD, a condition that is known to affect WBV [1]. Individuals affected by SCD have erythrocytes that take on a characteristic sickle shape when deoxygenated instead of the normal biconcave shape. To facilitate the study of the full range of behavior of sickle cell erythrocytes, Dr. Lykotrafitis's lab is equipped to conduct experiments under both oxygenated and deoxygenated conditions. Consequently, the proposed device should be operable in both an oxygenated and a deoxygenated environment.

The client has indicated the relevance of the device not only to his own research but to public health as well. The impacts of SCD are widespread; the disease occurs in >200,000 births per year in Africa and between 60,000 and 100,000 births per year elsewhere [2]. Patients with SCD exhibit a range of symptoms including chronic pain, frequent infections, necrosis, organ damage, and stroke [3][4]. The significance of altered blood rheology on the prognosis and the need for preventative care in SCD is established [5]. Commercial rheometers currently utilized for the measurement of WBV, however, involve methods that are performed using expensive equipment, are time consuming, require a large blood sample on the order of milliliters, and are not practical outside of a laboratory setting [6]. This may present a barrier to treatment, particularly in those areas in which individuals do not readily have access to healthcare and/or medical facilities. An ideal device would offer a simple, portable, inexpensive means to measure WBV.

The proposed device has clear applications to the measurement of WBV in SCD. The clinical relevance of the device, however, extends beyond this scope. Abnormal WBV is a means for early diagnosis, prognosis, and prevention in many other diseases [5]. In patients with cardiovascular disease, increased WBV has been correlated with an increased risk of cardiovascular events [7]. Cardiovascular disease, a diagnosis which encompasses conditions such as coronary heart disease, deep vein thrombosis, and peripheral arterial disease, results in more annual deaths worldwide than any other cause [8]. According to the World Health Organization, this trend is expected to continue in the future [8]. Like SCD, many of the individuals significantly impacted by cardiovascular disease live in areas with barriers to proper care, with 80% of the annual deaths resulting from the condition occurring in low- to middle-income countries [8]. Monitoring WBV shows promise as a point-of-care measurement in patients with cardiovascular disease and the application of the proposed device for this purpose is but one example of the relevance of the device beyond the scope of SCD [7].

### *1.2 Purpose*

Aim: **The goal of this project is to design, fabricate, and assess the performance of a simple and preferably inexpensive device that can measure WBV and its changes over time.**

It has been established that WBV is increased in patients with cardiovascular disease as well as in patients with sickle cell anemia [1][7]. The viscosity of blood can also vary throughout

the course of procedures such as cardiopulmonary bypass. Here, the aim is to design, fabricate, and test a simple device that can accurately measure the viscosity of whole blood. The client has stated that such a device should be easy to operate, inexpensive to fabricate, and preferably usable outside of a laboratory setting. Considering that the client is particularly interested in measurements of blood viscosity in SCD, the device will be designed to perform in both an oxygenated and deoxygenated environment.

### *1.3 Previous Work Done by Others*

Our optimal design uses a microfluidics approach to the design of the device. We are aware of two research teams currently investigating microfluidics-based viscometers for use with non-Newtonian fluids such as whole blood. Kang, et al. have designed viscometers for the measurement of WBV that utilizes a microfluidic device in conjunction with a syringe pump [9][10].

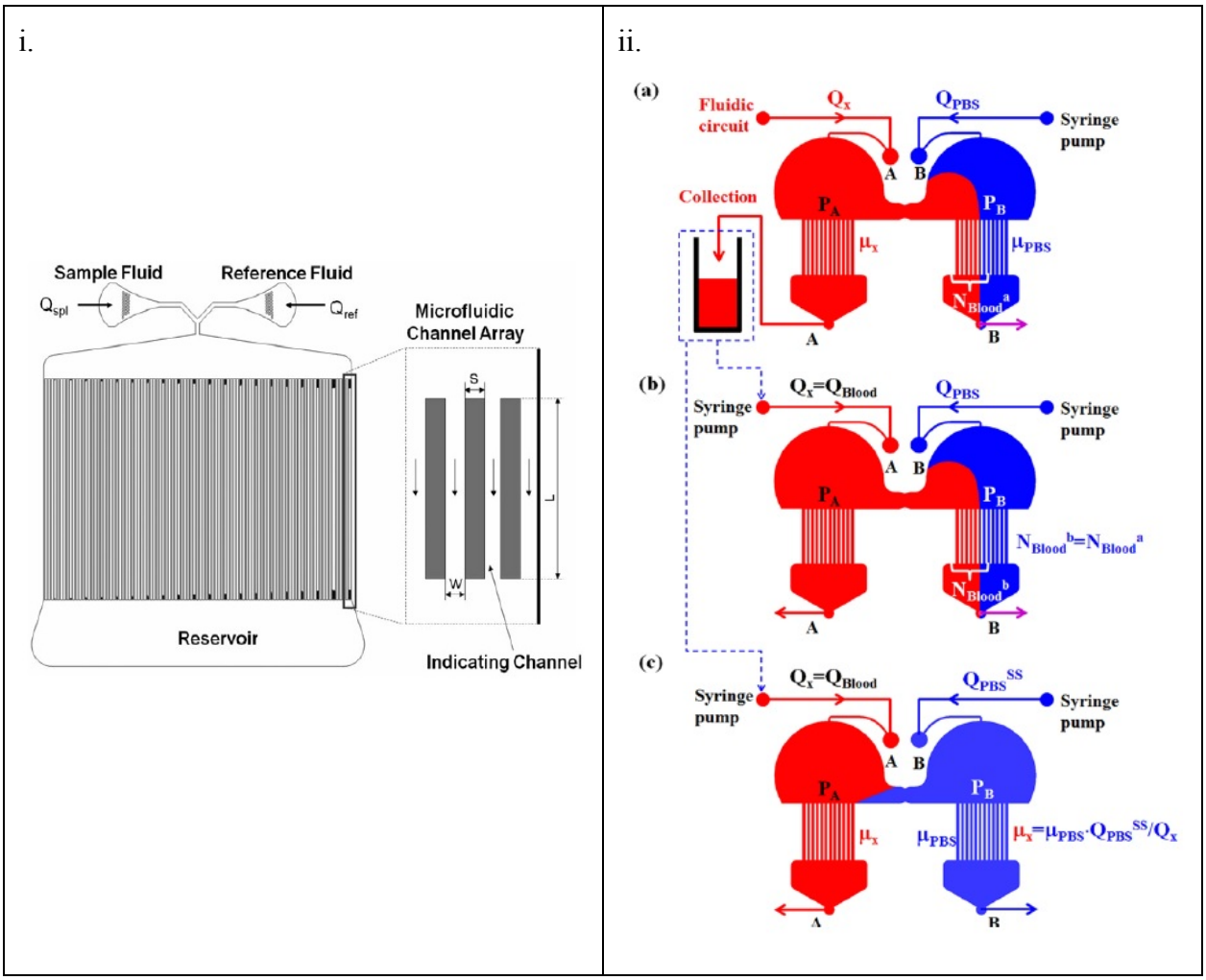


**Fig. 1**. i. Microfluidic device by Kang, et al. for the measurement of WBV [9] ii. Microfluidic device by Kang, et al. for the simultaneous measurement of flow rate and WBV in a complex fluidic network [10].

Figure 1 shows two microfluidics devices designed by Kang, et al. The device shown in Fig. 1 i. utilizes a microfluidic device featuring two inlets for the sample and reference fluids and a number of indicating channels. The length, width, height, and distance between indicating channels are L=10 mm, W=50 μm, h=60 μm, and S=100 μm, respectively [9]. The sample fluid for which the viscosity is to be determined and and a reference fluid of known viscosity are pumped into the inlets at known flow rates $Q_{spl}$ and $Q_{ref}$ [9]. Due to the fact that the pressure drop across the channels is identical, according to Poiseuille's law, the relative viscosity of the sample to the reference fluid may be calculated using the equation:

(1)

$$\mu_r = \left(\frac{\mu_{spl}}{\mu_{ref}}\right) = \left(\frac{N_{spl}}{N_{ref}}\right)\left(\frac{Q_{ref}}{Q_{spl}}\right)$$

where $\mu_{spl}$ and $\mu_{ref}$ are the viscosity of the sample and reference fluids, and $N_{spl}$ and $N_{ref}$ are the number of indicating channels filled with the sample and reference fluids [9]. Clearly, if $\mu_{ref}$ is known, $\mu_{spl}$ may be calculated. Further, using $Q_{spl}$, $N_{spl}$, W, and h, the shear rate may be calculated using the equation:

(2)

$$\dot{\gamma} = \left(\frac{6Q_{spl}}{Wh^2N_{spl}}\right)\left(\frac{1-\frac{192h}{\pi^5 W}\sum_{n=1,3,5,\ldots}\frac{1}{n^5}\tanh\left(\frac{n\pi W}{2h}\right)}{1-\frac{16h}{\pi^3 W}\sum_{n=1,3,5,\ldots}\frac{1}{n^3}\tanh\left(\frac{n\pi W}{2h}\right)}\right)$$

Fig. 1 ii. shows a second device designed by Kang, et al. that utilizes a similar technique to measure the viscosity of whole blood. This device adds the ability to measure WBV of a sample at an unknown flow rate $Q_x$ [10]. As shown in Fig. 1 ii., the device consists of three microfluidic channel arrangements. Similar to the first device, there are inlets for sample and reference fluids and microfluidic channels of width and depth W=300 μm and D=50 μm. During operation, the sample flows into the left inlet in Fig. 1 ii.a at flow rate $Q_x$ while the reference fluid is pumped into the left inlet at known flow rate $Q_{pbs}$ such that the sample enters the upper right chamber. Reference fluid is pumped into the right inlet of the second microfluidic arrangement (Fig. 1 ii.b) at known flow rate $Q_{pbs}$ equal to that of the first microfluidic arrangement. The sample is pumped into the left inlet (Fig. 1 ii.b) at flow rate $Q_{blood}$ such that the number of channels filled with blood ( $N_{blood}$) is equal to that of the first microfluidic arrangement. Consequently, it may be concluded that $Q_{blood}=Q_x$, allowing the unknown flow rate of the sample to be calculated [10]. The sample is then pumped into the left inlet of the third microfluidics arrangement at flow rate $Q_x=Q_{blood}$ as shown in Fig. 1 ii.c. The reference fluid is pumped into the right inlet (Fig. 1 ii.c) until the flow reverses at some flow rate $Q_{pbs,ss}$ and the reference fluid flows into the left half of the device. Due to the fact that the fluidic resistance in each of the devices is identical, the unknown viscosity of the sample may be calculated using the equation:

(3)

$$\mu_x = \frac{\mu_{pbs} \cdot Q_{pbs}}{Q_{blood}}$$

Additionally, using $Q_{blood}$ and the hydraulic diameter of the channels indicated by D and N, the shear rate of the sample in the channels may be calculated using the equation:
(4)

$$\dot{\gamma} = \frac{32Q_{blood}}{\pi N D^3}$$

A different approach for the measurement of WBV has been developed by Srivastava, et al. and Srivastava and Burns [11].

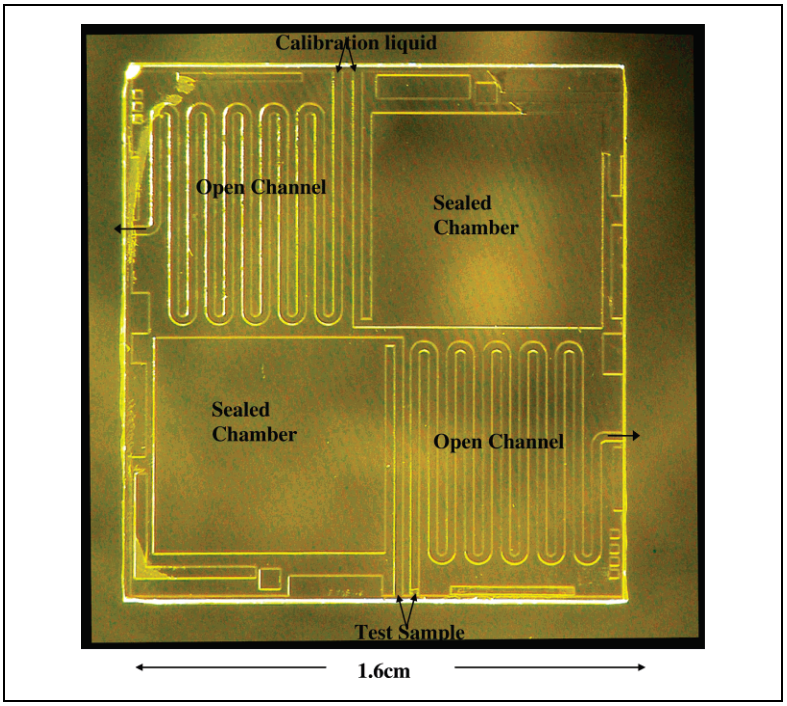


**Fig. 2.** Self-calibrating microfluidic device for measurement of WBV by Srivastava, et al. [11].

Figure 2 depicts a self-calibrating microfluidic device for the measurement of WBV designed by Srivastava and Burns [11]. The device exhibits two identical open microfluidic channels of width, length, and depth W=400 μm, L=8 cm, and D=100 μm and two identical arrangements of a microchannel joined to a sealed chamber. A sample and reference fluid are placed at the inlets as indicated by the labels and arrows in Fig. 2. Capillary pressure draws the fluids into the open channels and the velocity of the fluid through the channel is monitored. The capillary pressure $P_{capillary}$ is determined using the microchannels joined to the sealed chambers. According to the ideal gas law,
(5)

$$P_{capillary} = P_{atm}(\frac{V_1}{V_2} - 1)$$

where $P_{atm}$ is the atmospheric pressure and $V_1$ and $V_2$ are the original and compressed volumes of air in the sealed chamber, calculated based on device dimensions and the position of

the fluid meniscus [11]. The unknown viscosity of the sample may be calculated using the equation:
(6)

$$\mu_{sample} = \mu_{cal}\left(\frac{vL_{cal}}{P_{capillary,\,cal}}\right)\left(\frac{P_{capillary,\,sample}}{vL_{sample}}\right)$$

where $\mu_{sample}$ is the sample viscosity, $\mu_{cal}$ is the calibration fluid viscosity, $vL_{cal}$ and $vL_{sample}$ are the velocity multiplied by the length traveled by the calibration and sample fluids, respectively, and $P_{capillary,\,cal}$ and $P_{capillary,\,sample}$ are the calculated capillary pressures of the calibration and sample fluids.

### *1.3.1 Products*

We are aware of no products similar to the proposed device outside of the aforementioned research conducted by Kang, et al. and Srivastava and Burns. No commercially available devices similar in nature were found.

### *1.3.2 Patent Search Results*

- Burns, et al. United States Patent. Patent No. US 7,188,515 B2. Nanoliter viscometer for analyzing blood plasma and other liquid samples.
- Chien, et al. United States Patent. Patent No. US 6915679 B2. Flexible, selective transportation of fluids within microfluidic channels of a microfluidic network by controlling pressures at reservoirs; fluid flows through the channel segments resulting from a pressure can be determined
- Kensey, et al. United States Patent. Patent No. US 6745615 B2. Dual riser/single capillary viscometer.
- Minton, et al. United States Patent. Patent No. WO 2014031639 A1. Capillary viscometer and multiscale pressure differential measuring device.
- Spaid, et al. United States Patent. Patent No. US 7040144 B2. Microfluidic viscometer.
- Spaid, et al. United States Patent. Patent No. US 6990851 B2. Microfluidic viscometer.
- Spaid, et al. United States Patent. Patent No. US 6681616 B2. Microfluidic viscometer
- Varni, et al. United States Patent. Patent No. WO 2013066806 A1. Automated capillary viscometer.

### *1.4 Report Map*

The following report details the optimal design chosen for the device sought by the client. Eliminated alternatives are described and details are given on the design of each of the components of the chosen device, the rationale, and proposed methods for testing of the design. The mathematical framework for the device is outlined and the fabrication methods are discussed. The realistic constraints of the project and safety issues are considered, and the budget and timeline of the project are detailed. Reflections on the effects of the proposed engineering solutions and the impact on lifelong learning are included.

## 2. Project Design

### *2.1 Introduction*

The proposed design utilizes a microfluidic platform to determine the unknown viscosity of a blood sample over a range of shear rates that are reflective of the conditions present in the human body. The device utilizes capillary pressure to drive the flow of the sample through serpentine microchannels of varied width, and using the determined value of the capillary pressure, channel dimensions, and sample velocity, the viscosity of the sample can be calculated. The proposed design is supported through mathematical theory, and the supporting equations have been previously used in the operation procedure of microfluidic viscometers. The device proposed here will be fabricated utilizing a wet chemical glass etching method that has been previously used in the fabrication of microchannels.

The selected design was chosen over an originally proposed device that operated on the same principle but differed only in the number of channels and the microchannel geometry. Our original design included only straight channels: two sets of closed channels; and two sets of open channels identical in geometry to those of the closed channels but with two open ends. We chose our optimal design over our original design since the use of serpentine channels of varied width versus multiple straight channels allows us to decrease the size of the device and simplify the procedure for device operation by using only one sample in the open channels. Additionally, the use of fewer channels decreases the size of the blood sample needed from the patient.

A second alternative device was proposed based upon the phenomenon of electrowetting. This alternative microfluidic device for measuring whole blood viscosity uses electrowetting as the mechanism to facilitate the flow of the non-Newtonian blood sample. Electrowetting is a phenomenon in which the application of an electric field alters the surface tension and essentially pulls the droplet down onto the electrode, therefore decreasing the contact angle and increasing the droplet contact area [12][13]. These changes in surface properties induce droplet motion. The application of such an electric field can be utilized to drive a droplet of fluid through a microfluidic channel. In a study by Lin, et al., electrowetting on dielectric (EWOD) is adopted to drive liquids. Variously sized electrodes on a chip are employed to measure the velocity gradient to determine the viscosity coefficient. The device is fabricated by microelectromechanical system (MEMS) technology [14]. Based on theories regarding viscometry and electrowetting described by Lin, et al., electrowetting on dielectric (EWOD) was chosen as the optimal method of electrowetting for the purposes of this research. EWOD was used to drive the flow through the channels of the device by altering the contact angle, surface tension, and the electron distribution of the dielectric. The external force exerted by EWOD is:

(7)

$$F_{ex} = \Delta p \Delta A_{cross} = \frac{\varepsilon_o \varepsilon V^2}{2d_t} \Delta A_{cross}$$

where ΔAcross is the cross-sectional area of the channel. In the linear channel, the viscosity coefficient relates to the external force by:

(8)

$$F_{ex} = S_1 \Delta\Delta A_{cross} = \eta\left(\frac{\partial v_x}{\partial y} + \frac{\partial v_y}{\partial x}\right)\Delta A_{tan}$$

where ΔAtan is the tangent area in contact with the liquid and channel wall. A variety of physical considerations influence equation 2 including channel size, dielectric constant of the material,

thickness of the material, and size of the electrode. From equations 1 and 2, the linear channel viscosity coefficient $\eta$ l can be determined by:
(9)

$$\eta_l = \frac{1}{\frac{\partial v_x}{\partial y}+\frac{\partial v_y}{\partial x}} \frac{\varepsilon_o \varepsilon V^2}{2d_t} \frac{\Delta A_{cross}}{\Delta A_{tan}}$$

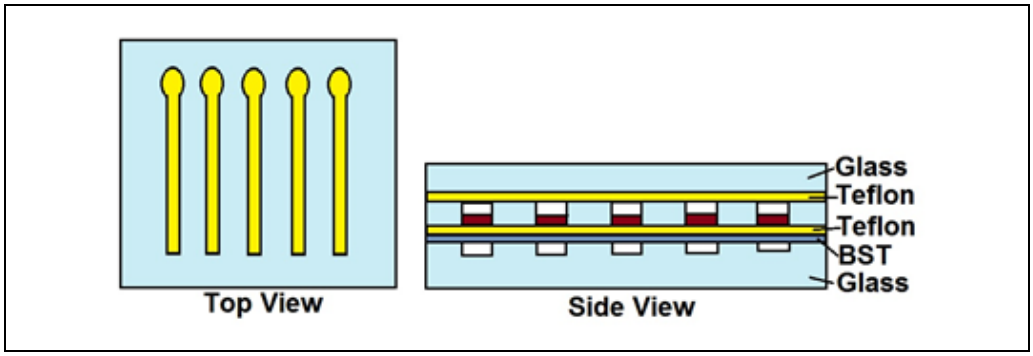


**Fig. 3.** Top and cross-section view of the EWOD microfluidic device.

Based on the theory defined in Lin, et al., we composed this alternative design as presented in Fig. 3, which displays a top view and a cross-section view. In our design we created five separate channels so the blood viscosity of 5 different samples can be measured at a time. The five blood samples can be pipetted near the inlet and the mechanism of electrowetting will draw the sample into the channel. The cross-section view shows the various layers used to create the electrowetting on dielectric device. In this design we are using barium strontium titanate (BST) as the dielectric material as it has a high dielectric constant. The electrode surfaces are coated with teflon which is hydrophobic in the absence of an electric field but becomes hydrophilic when an electric field is applied.

Although this is plausible method for measuring viscosity, there is minimal literature on the application of electrowetting for measuring non-newtonian fluid viscosity. Therefore, we felt that using electrowetting for measuring WBV would have required extensive background research and experiments not feasible within the limited time frame we were given. In addition, the fabrication method for creating a microfluidic device with electrowetting is complex and requires processes and equipment which are not available to us, thus replicating these methods would be difficult. Additionally, compared with the cost of our chosen design, electrowetting is the more expensive option. Our chosen optimal design better satisfies the client objective of an inexpensive device.

The final alternative design is an extension of previous work done by Kang et al.

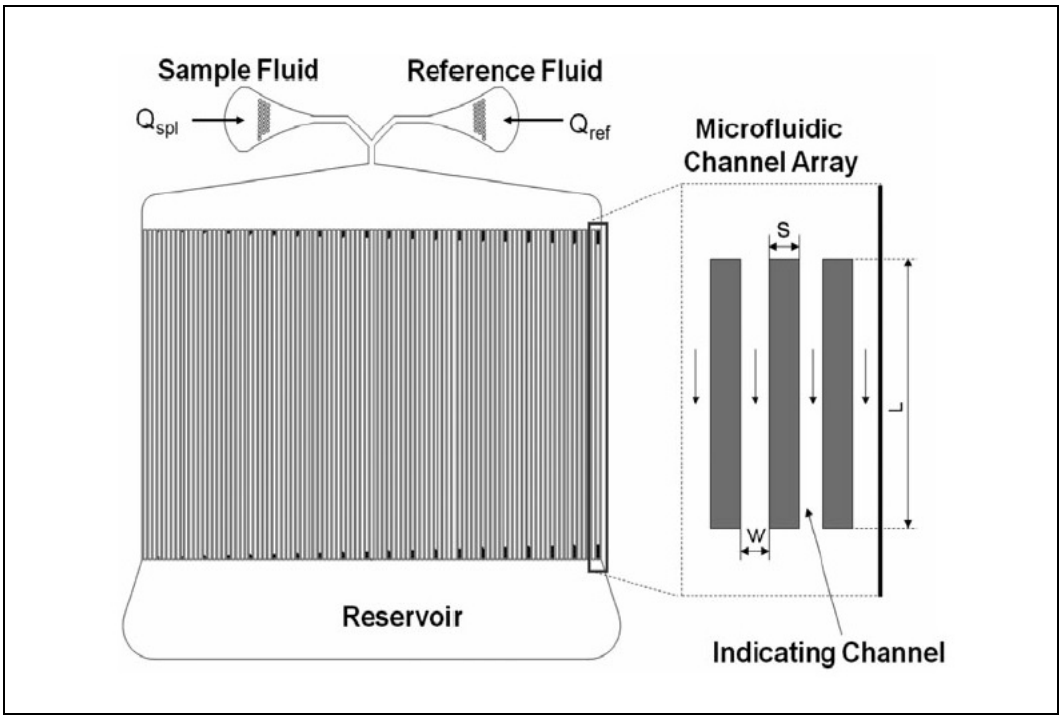


**Fig. 4.** Microfluidic device for measurement of whole blood viscosity designed by Kang et al. [9].

The device utilizes a microfluidic channel array with two inlets for a blood sample and a reference fluid of known viscosity. The sample and reference fluids are pumped via a syringe pump into the microfluidic array at known flow rates $Q_{spl}$ and $Q_{blood}$. Due to the fact that the pressure drop across all of the channels is the same as a result of their identical dimensions, the relative viscosity of the sample to the reference fluid may be calculated as:

(10)

$$\mu = \frac{\mu_{spl}}{\mu_{ref}} = \frac{N_{spl} Q_{ref}}{N_{ref} Q_{spl}}$$

where $\mu_{spl}$ and $\mu_{ref}$ are the sample and reference fluid viscosities, $N_{spl}$ and $N_{ref}$ are the number of microchannels filled with the sample and reference fluids, and $Q_{spl}$ and $Q_{ref}$ are the sample and reference fluid flow rates. It can be seen from equation 6 that the unknown viscosity of the blood sample may be calculated since the flow rates, reference fluid viscosity, and number of filled microchannels are known.

While the described device provides a simple mechanism for the measurement of blood viscosity, syringe pumps are both costly and have little portability. Here we propose the use of a microfluidic gravity pump to move the fluid through the microfluidic array.

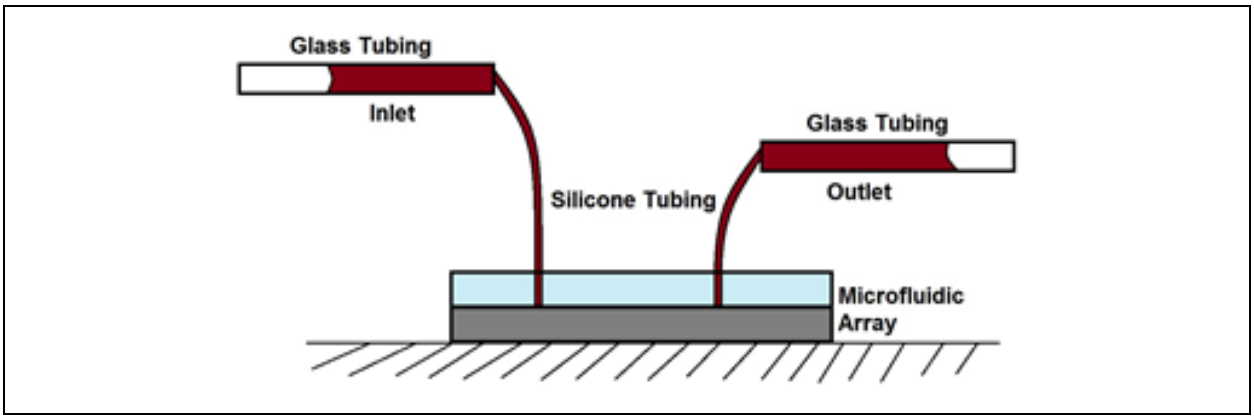


**Fig. 5.** Gravity pump design using glass tubing as a fluid reservoir. Fluid enters microfluidic array via silicone tubing [15].

Zhu et al. have developed a microfluidic gravity pump that delivers fluids at a consistent flow rate (Fig. 5) [15]. The benefits of utilizing a similar design in place of a syringe pump as applied to the microfluidic viscometer designed by Kang, et al. include increased portability and cost efficiency of the device. This is particularly suited to the objectives set forth by our client. We thus propose utilizing two gravity pumps with inlets as shown in Fig. 4, with an additional two outlets at the corners of the reservoir depicted at the bottom corners of the device (Fig. 4). The proposed device would be fabricated using the soft lithography method described in our original device design.

While the device allows for the measurement of WBV over a clinically meaningful range of shear rates, the simplicity and cost-effectiveness of the device are limited by the incorporation of gravity pumps. Ultimately, the chosen optimal device design was determined to best meet the client's objectives of simplicity, low cost, and portability.

### *2.2 Prototype*

#### *2.2.1 General Overview of Device*

*Microchannels:*

The microfluidic rheometer design is composed of serpentine and sealed microchannels of varying dimensions designed to measure WBV over a range of shear rates that is clinically meaningful. Using the known values of the channel dimensions, the capillary pressure, and the velocity of the sample traveling through the microchannels, the viscosity of the sample may be calculated [1][2]. The overall device consists of two sets of serpentine open channels of varying widths and two sets of closed channels. One set is used for the reference fluid of known viscosity and the second set is used for the blood sample of unknown blood viscosity.

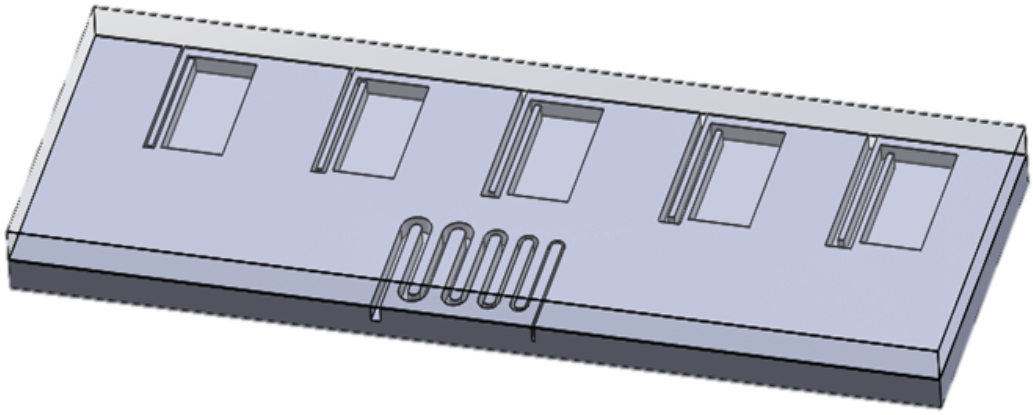

**Fig. 6**. Depiction of the open serpentine channels and closed channels of the device.

A schematic of the design of the device is provided in Fig. 6. The open serpentine channel is shown at the bottom of the device, while the closed channels are shown at the top of the device. The microchannels of the device are fabricated using a wet chemical etching method on glass slides. The etched glass slides are bonded to a plain glass substrate using optical glue to complete the device. The accuracy of the microfluidic rheometer was tested versus a parallel plate rheometer. The device is simple to use, portable, operable in an oxygenated or a deoxygenated environment, and costs approximately $1.40 per device. The proposed device is novel as compared to other microfluidic viscometers in that it utilizes no external pumping mechanism or power while providing a measurement of whole blood viscosity over a range of shear rates found in the human body.

### *2.2.2 Subunits*

*Microfluidic Channels:*

*Description and Theoretical Justification:*

Our device builds upon the research of Burns, et al. and Burns and Srivastava [11][16]. The device consists of four microfluidic channels: two identical sets of open serpentine channels of varied width; and two identical sets of closed channels of widths identical to those of the serpentine channels. To determine the unknown viscosity of a blood sample, both the sample and a reference fluid of known viscosity need to be utilized. One set of each of the open and closed channels are used with the blood sample and the reference fluid.

The fluid flow within microfluidic channels is characterized as laminar flow. Within the channels of the microfluidic device fluid flows in parallel lines with no disruption between the flow lines. All the layers of the fluid flow in a single direction with no perpendicular cross current creating a smooth and orderly fluid flow. The force required to move a layer of fluid against another is defined as dynamic viscosity. The measurement of dynamic viscosity obtained from our microfluidic viscosity is based on the Hagen-Poiseuille law. The Hagen-Poiseuille law describes the relationship between the pressure difference and fluid flow in a cylindrical tube under steady flow conditions [18]. Fluid flow is defined by the following equation:

(11)

$$Q = \frac{\Delta P * \pi * r^4}{8 * \mu * l}$$

where ΔP is the pressure difference across the channel, r is the radius of the tube, l is the length of the tube, μ is the fluid viscosity, and Q is the fluid flow. While the Hagen-Poiseuille law is typically applied to Newtonian fluids, it has previously been established in research that this equation may be extended to describe the behavior of non-Newtonian fluids such as blood in microchannels [9][10][11][16]. Thus it may be assumed that the Hagen-Poiseuille law can be extended to include non-Newtonian viscosity measurements using the microfluidic device presented in Fig 6. In addition to the Hagen-Poiseuille law, a second mathematical model will be applied to characterize the viscosity of whole blood using the proposed device. The power law expression is a generalized Newtonian model which can be applied to a non-Newtonian fluid. The power law expression is defined by the following equation:
(12)

$$\eta = m * \dot{y}^{n-1}$$

where η is the non-Newtonian viscosity, m is a constant characterizing the fluid, ẏ is the shear rate and n is a constant which characterizes a fluid as either shear thinning or shear thickening [16]. The combination of the power law expression and the Hagen-Poiseuille law provides the complete mathematical model for the measurement of non-Newtonian viscosity utilized in the proposed optimal design.

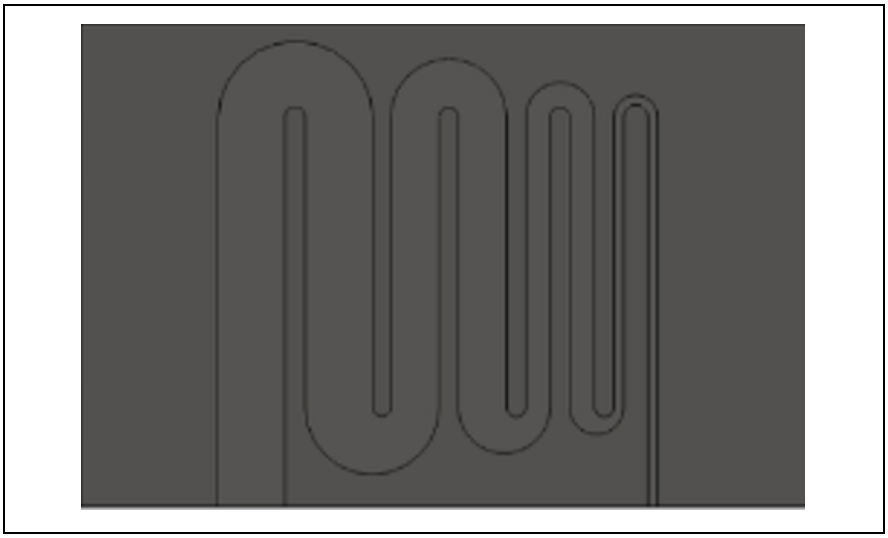

**Fig. 7.** Open serpentine channel configuration.

The open channel segments of varied widths (Fig. 7) allow for more clinically meaningful data by enabling the measurement of blood viscosity over a range of shear rates present in the human body. Additionally, by fabricating one channel of varying width versus multiple channels, a smaller blood sample may be used.

Our device uses the physical phenomenon of capillary pressure to drive fluid flow through microfluidic channels. This option facilitates the construction of a microfluidic device

that requires no external pumping mechanism or energy source and is thus an ideal choice to meet the objectives of portability and cost-efficiency established by our client. The capillary pressure in a microfluidic channel is governed by the channel geometry, the contact angles of the fluid against the channel walls, and the surface tension of the fluid. This relationship may be described mathematically via the following equation:

(13)

$$P_c = -\gamma\left(\frac{\cos\theta_b+\cos\theta_t}{h} + \frac{\cos\theta_l+\cos\theta_r}{w}\right)$$

where $P_c$ is the capillary pressure, $\gamma$ is the surface tension of the fluid, h is the height of the microchannel, w is the width of the microchannel, and $\theta_b$, $\theta_t$, $\theta_l$, and $\theta_r$ are the contact angles of the fluid on the bottom, top, left, and right channel walls, respectively [17]. It may be seen in equation (13) that the capillary pressure is independent of the length of the microchannel. This fact was exploited in our design, allowing us to use a single open serpentine channel of varying widths for viscosity measurement while using shorter channels of varied widths to separately determine the capillary pressure of the open channel needed for calculation of viscosity.

**Fig. 8.** Closed channel configuration.

The capillary pressure of both the sample and reference fluids is calculated using the closed channels shown in Fig. 8. The fluid is applied at the inlet of each of the closed channels. One set of channels is used for the blood sample, and the other identical set of channels is utilized with the reference fluid. According the the ideal gas law, here the capillary pressure may be determined by:

(14)

$$P_{capillary} = P_{atm}\left(\frac{V_1}{V_2} - 1\right)$$

where $P_{atm}$ is the atmospheric pressure, $V_1$ is the volume of air originally in the channel, as calculated using the channel dimensions, and $V_2$ is the volume of air in the channel after the sample is added, calculated from the location of the air-fluid meniscus and the channel dimensions. The samples are then applied to the inlets of the open channels. The viscosity of the unknown sample is equal to:

(15)

$$\eta = \frac{d^2}{s}\frac{1}{\frac{2}{3}+\frac{1}{3n}}\frac{\Delta P}{vL}$$

where $\Delta P$ is equal to the capillary pressure of the blood sample, and the other variables are calculated as follows:

- $\frac{d^2}{s} = \mu_{calib}\frac{vL_{calib}}{P_{capillary,calib}}$ , where $\mu_{calib}$ is the known viscosity of the reference fluid, $L_{calib}$ is the length traveled by the reference fluid in the open channel, v is the velocity of the calibration fluid in the open channel which is equal to $L_{calib}$ divided by the time for fluid travel, and $P_{capillary,\,calib}$ is the capillary pressure of the calibration fluid, calculated using equation 4;

- L is the length of the blood sample in the open channel;

- v is the velocity of the blood sample in the open channel, equal to $\frac{L}{t}$, where L is the length of the blood sample in the open channel and t is the time for the sample to travel the length L;
- n is found from a log-log plot of $\frac{1}{L}$ vs. velocity (v). The value of n is less than 1 for shear thinning fluids and greater than 1 for shear thickening fluids.

*Deoxygenation Chamber:*
*Description and Theoretical Justification:*

When measuring the WBV of blood samples taken from SCD affected patient a deoxygenation chamber is used to observe the sickling behavior of red blood cells under deoxygenated conditions. The deoxygenation chamber will be used both while using the microfluidic device and with the conventional parallel-plate rheometer to confirm the accuracy of viscosity measurements. The deoxygenation chamber is shown in Fig. 9. There are two top cover components which can be used with the chamber. One is a split cover with a circular opening in the center which is to be used with the parallel plate rheometer and the other is a solid cover to be used with the microfluidic device.

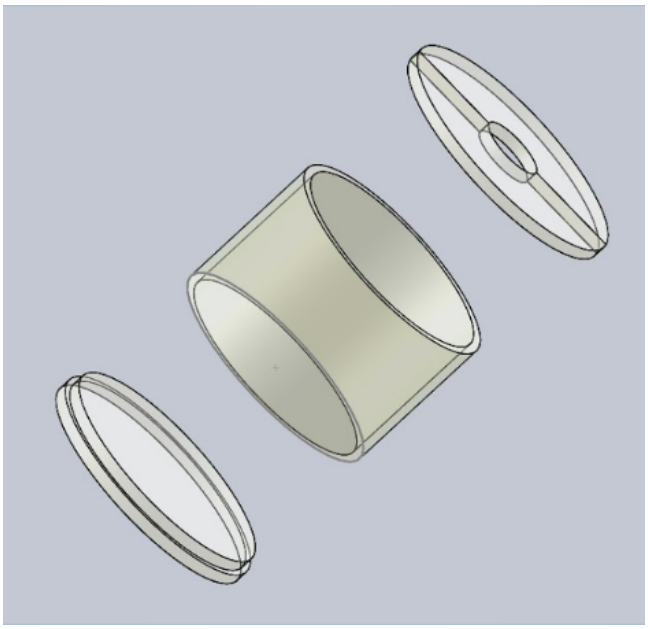

**Fig. 9.** Exploded view of the deoxygenation chamber design. Split cover is shown to the upper right, the wall section if shown in the middle, and the solid cover is shown to the lower left.

The sides of the chamber are constructed from polycarbonate (PCB) tubing. The top and bottom portions of the chamber are constructed from PCB sheeting. As previously stated the chamber has one split and one solid cover. The split cover has a hole in the middle when the two halves are joined. This allows the cover to be placed around the shaft of the parallel-plate rheometer geometry to best seal out the oxygen from the surrounding environment. The halves of the split cover secure into the cylindrical portion of the deoxygenation chamber and the solid cover also seals the chamber in a similar way. The PCB material used to manufacture the chamber has low bioreactivity and low reactivity with nitrogen, thus maintaining the deoxygenation of the blood samples.


**Fig. 10.** Rheometer used in combination with deoxygenation chamber.

### *2.2.3 Fabrication Protocols*

***Protocol: Photoresist Masking of Slides***

*Materials and Equipment:*

- spin coater
- Fume hood
- Photomask (500 dpi resolution)
- Mask aligner/UV light source
- Pre-cleaned glass slides
- PR-4000A photoresist (Futurrex, Inc.)
- RD-6 photoresist developer (Futurrex, Inc.)
- Tray or pan for developing
- Hot plate
- Distilled water

1. Set the hot plate to 90℃

2. Place a glass slide onto the spin chuck of the spin coater and vacuum. Spin by hand to ensure that the slide is centered.

**Fig. 11.** Spin coater used to coat glass slides with an even layer of photoresist. (image: www.deanlab.com)

3. Program the spin coater to an initial step of 300 rpm for 5 s followed by a second step of 500 rpm for 20 s. Perform a trial run to ensure that the program is working properly.

4. After ensuring correct operation of the spin coater, pour enough photoresist onto the slide to coat it. Be sure to pour photoresist along entire length of the slide.

5. Close the lid of the spin coater and run.

6. When the spin coater is finished, open the lid quickly to avoid drips onto the slide. Turn off the vacuum, remove the slide and bake on the hot plate at 90℃ for 10 minutes. Remove from hot plate and cool. Increase the temperature of the hot plate to 100℃.

7. Align the photomask over the slide using the mask aligner.

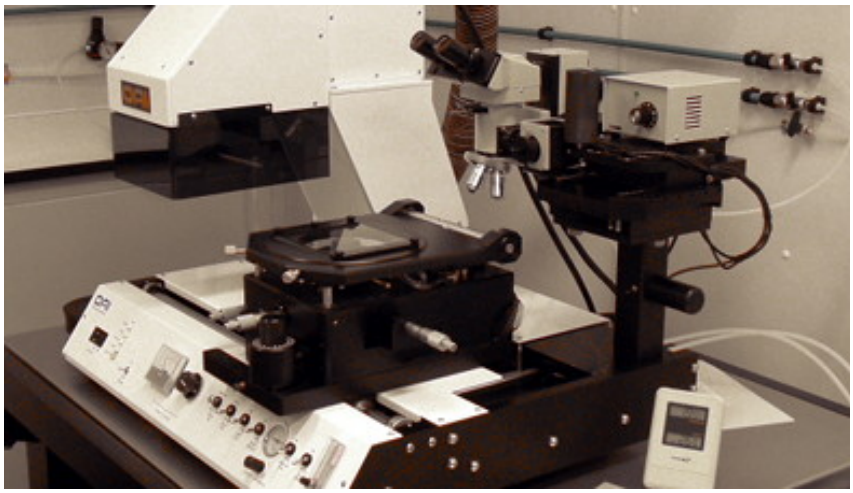

**Fig. 12**. Mask aligner used for proper positioning of photomask during UV exposure. (image: www.nanoqam.uqam.caimagesdevices36 )

8. Expose the slide to UV light for 10 s.

9. Immediately following UV exposure, bake the slide for 3 minutes at 100℃ on the hot plate. Remove from hot plate and cool. Increase the temperature of the hot plate to 130℃.

10. Place the slide into the tray or pan and add and amount RD-6 developer sufficient to cover slide. Gently swirl for 3.5 minutes.

11. Remove slide and rinse with distilled water. Rinse the tray with distilled water and empty into the proper disposal container.

12. Bake the slide on the hot plate at 130℃ for 90 minutes. Remove and cool.

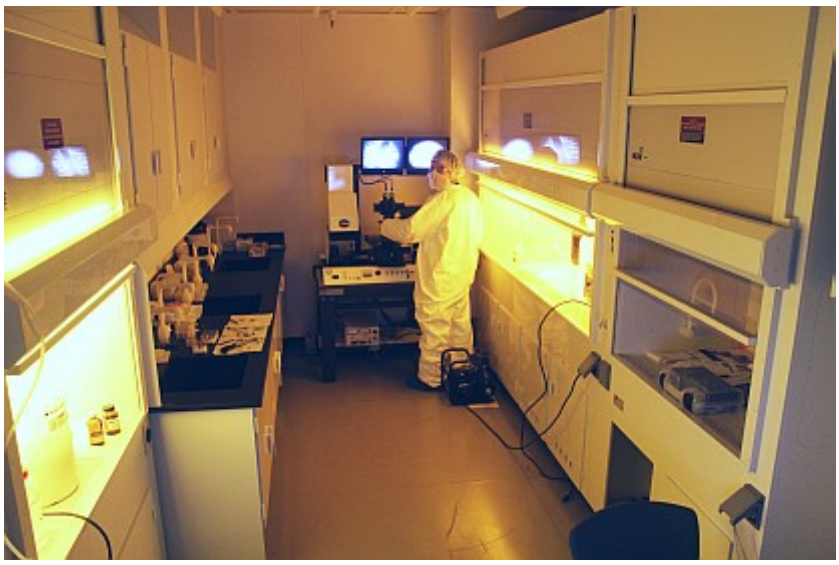

**Fig. 13.** Clean room facility in which the slides are spin coated, UV exposed, and developed. (image: www.biotech.uconn.edu )

***Protocol: Wet Chemical Etching of Glass Slides***

**DANGER:** Use of hydrofluoric and hydrochloric acid may result in severe injury and/or death. Proper training of personnel handling these materials is necessary. Acids must be handled only under a fume hood. Avoid breathing vapors as they may be lethal. A hydrofluoric acid spill kit including calcium gluconate gel should be on hand when using this protocol.

*Materials and Equipment:*

- Fume hood
- Nalgene beakers, 600 mL (5), 500 mL (1), 100 mL (2)
- Graduated cylinder
- Polyethylene tweezers
- Glass slides
- Distilled water
- Hydrofluoric acid
- Hydrochloric acid
- Acetone

1. Place one photoresist-masked slide photoresist side up in the bottom of each of the four 600 mL Nalgene beakers and place in the fume hood.

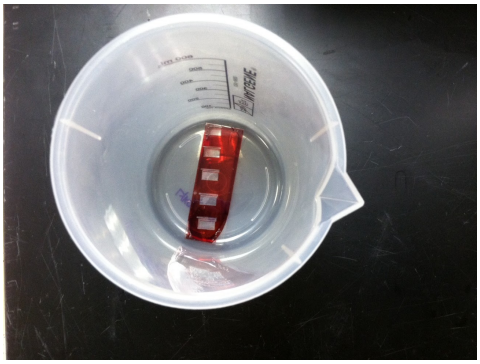

**Fig. 14**. Placement of photoresist-masked slide in Nalgene beaker for etching.

2. Using the graduated cylinder, measure 80 mL distilled water and pour into the 500 mL Nalgene beaker and place in the fume hood.

3. Pour approximately 300 mL of water into a 600 mL Nalgene beaker and place in the fume hood. This may be used for the disposal of waste acid if necessary.

4. Working under the fume hood, measure 20 mL of hydrofluoric acid into a 100 mL Nalgene beaker. If necessary, pour excess slowly into the 600 mL beaker containing the ~300 mL distilled water. Slowly pour the 20 mL of hydrofluoric acid into the 500 mL beaker containing the 80 mL distilled water.

5. Working under the fume hood, measure 40 mL of hydrochloric acid into the second 100 mL Nalgene beaker. If necessary, pour any excess slowly into the 600 mL waste beaker. Add the 40 mL hydrochloric acid slowly to the 500 mL beaker containing the distilled water and hydrofluoric acid.

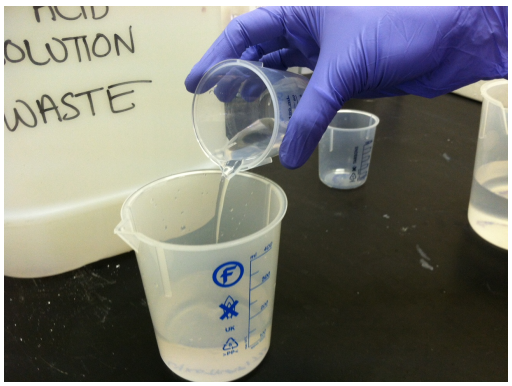


**Fig. 15.**. Pouring of acid into distilled water to prepare etching solution.

6. Carefully pour the etching solution over the glass slides in the 4 600 mL Nalgene beakers, dividing the solution approximately equally. Ensure that the solution is covering the slides as shown in Fig. 16. Gently swirl the beakers to get the slides to sit on the bottom if necessary. The solution will etch the glass at a rate of ~0.65 μm/minute. Here, an etching time of 46 minutes is used to attain a theoretical channel depth of ~30 μm.

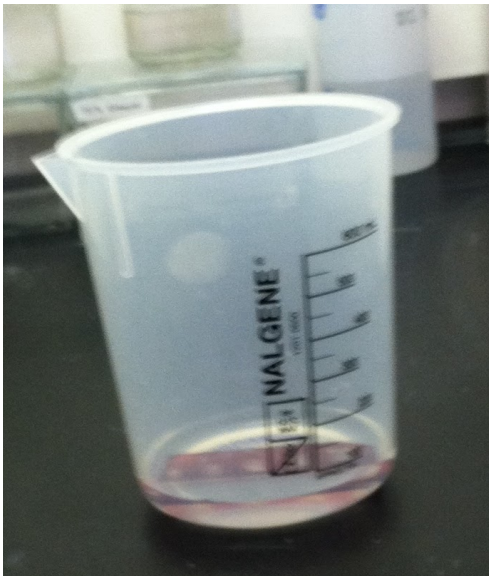


**Fig. 16.** Slide to be etched covered in etching solution. Approximately 35 ml of etching solution is used per slide.

7. Rinse the 100 mL and 500 mL Nalgene beakers with distilled water, disposing of the excess in the 600 mL waste beaker. Empty the waste beaker into the proper container between rinses if necessary, adding more distilled water to the beaker for disposal after emptying. Rinse the 600 mL beaker with distilled water.

8. Wash the distilled water-rinsed 100 mL, 500 mL, and waste beakers in the sink and let air dry.

9. Before removing the etched slides from the solution, fill the 500 mL Nalgene beaker with sufficient distilled water to cover slides and place in the fume hood. Prepare the waste beaker, adding ~300 mL distilled water to the 600 mL Nalgene beaker and place under the fume hood.

10. Remove the etched slides with the polyethylene tweezers and place into the 500 mL beaker to soak. Rinse the tweezers in the 500 mL beaker.

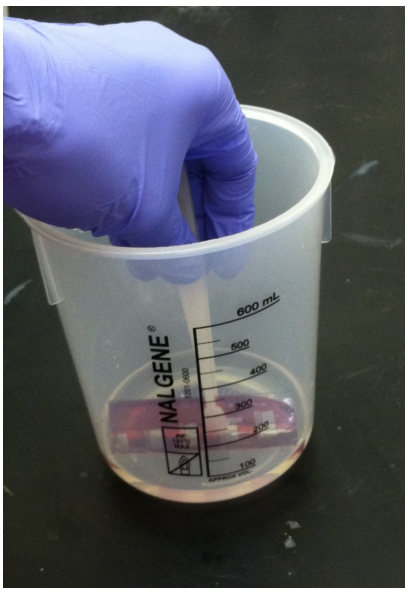


**Fig. 17.** Removing etched slide with polyethylene tweezers and slide soaking in beaker of distilled water to rinse.

11. Dispose of the etching solution in each of the 600 mL beakers by slowly pouring into the waste beaker. Empty the waste beaker as necessary, refilling with distilled water.

12. Remove slides from distilled water rinse and dry on a paper towel.

13. Rinse all beakers with distilled water.

14. Wash beakers in sink and let air dry.

15. Remove any remaining photoresist from slides using acetone and rinse with distilled water.

*Note:* This protocol provides etchant sufficient for four glass slides. Reagent amounts may be adjusted as necessary in a 4:2:1 ratio of distilled water:hydrochloric acid:hydrofluoric acid.

***Protocol: Bonding Etched Slides***

*Materials and Equipment:*

- UV glue, Lens Bond Type SK-9, 40 cps (Summers Optical)
- UV light source
- Etched slide
- Pre-cleaned glass slides

1. Place 3 drops of glue on one plain glass slide. This slide will be used only to aid in the even distribution of glue onto the slide to be bonded to the etched slide. Place a second glass slide onto the first slide. This will cause the glue to spread into a thin layer between the slides.

2. Remove the top slide by gently sliding it sideways off of the other slide.

3. Starting at the end of the etched slide opposite the inlet of the channels, gently place the glue-coated slide on top of the etched slide. Push the slide gently towards the inlets of the channels, ensuring that the only inlets remain open and that the plain slide covers the remainder of the etched channels. Ultimately, the slides will be staggered on top of one another as shown in Fig. 18.

**Fig. 18.** Alignment of the etched and plain slides during bonding. The plain slide is positioned such that only the inlets of the channel are not covered.

4. Inspect channels to ensure that the glue surrounds all areas and is not blocking any of the channels. Gently press on any areas in which the glue has not appeared to have travelled. Blocked channels will have portions that are not visible; this is where glue has filled the channels. If necessary, remove the top slide, clean off the glue with acetone, and repeat the process.

5. Once satisfied that the glue is properly distributed, place the device under the UV light source for one hour to complete bonding. The UV light source should be approximately 1 in. over the device.

### *2.2.4 Operation of Device*

Each completed device consists of two serpentine microchannels etched in glass and two sets of five microchannels in a closed channel configuration. One accessory which can be used with the device is a deoxygenation chamber.

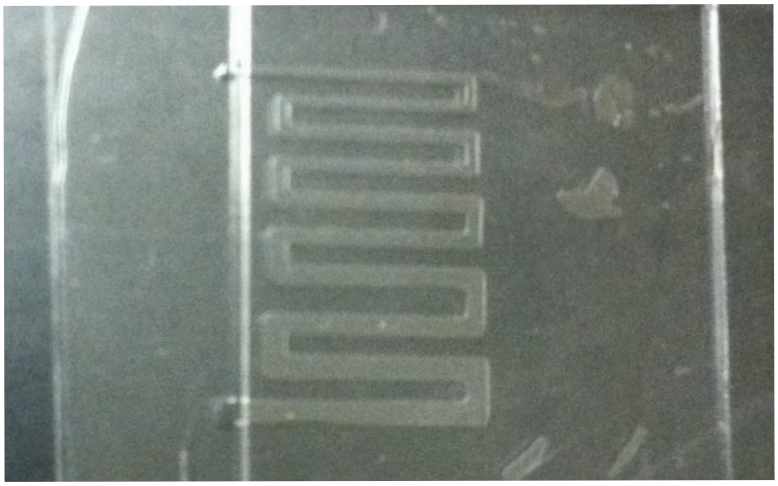

**Fig. 19.** Open serpentine microchannel. Each complete device includes of two of the channels pictured.

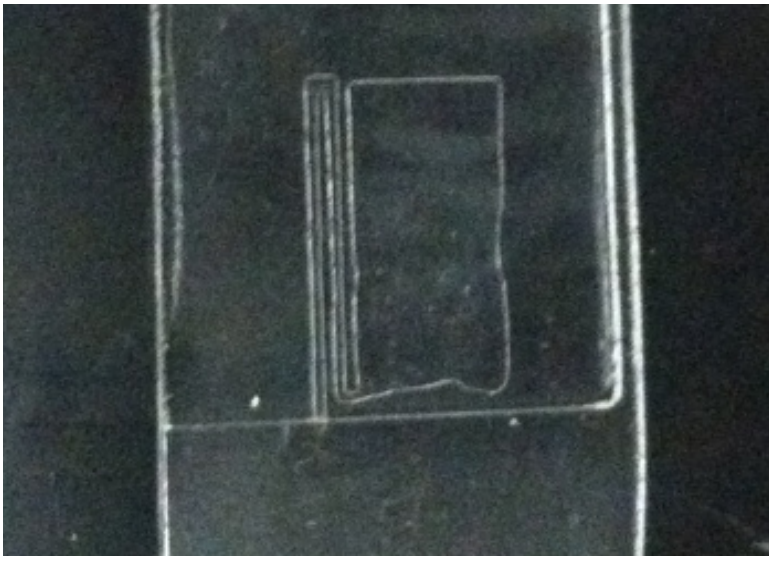

**Fig. 20.** One microchannel of the closed configuration. Each device includes two sets of five closed microchannels of varied width.

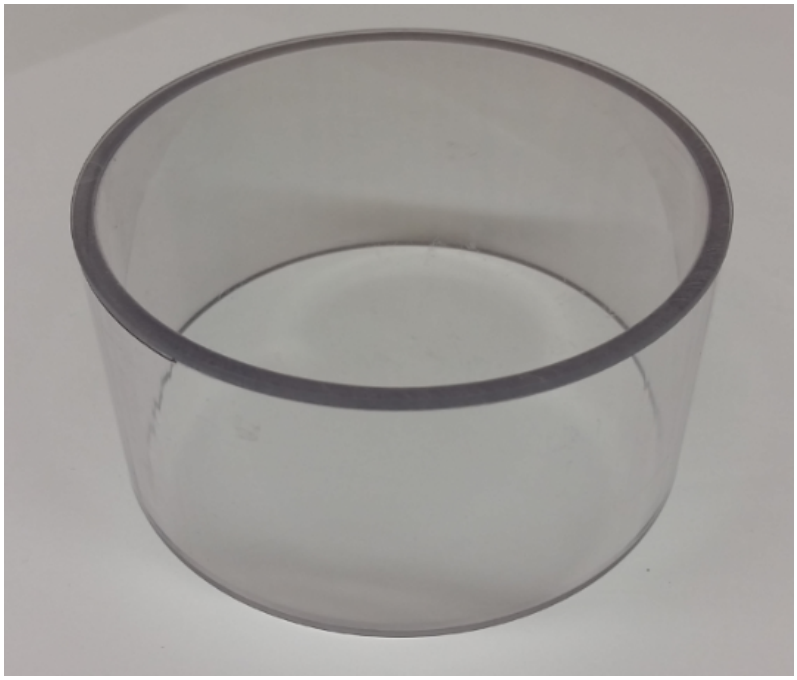

**Fig. 21.** Deoxygenation chamber shown with solid cover.

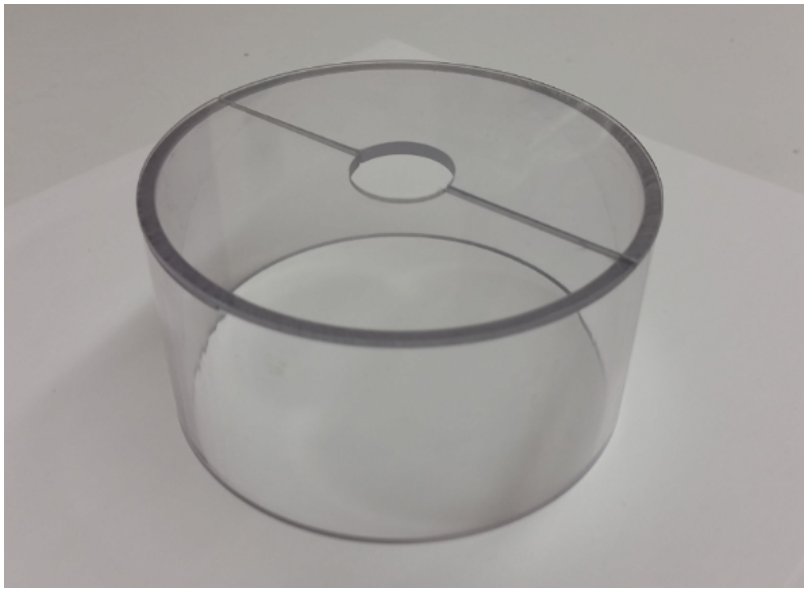

**Fig. 22.** Deoxygenation chamber shown for split cover suitable for use with a conventional parallel-plate rheometer.

*Operation of Deoxygenation Chamber with Microfluidic Device:*

The deoxygenation chamber is to be used with the inverted microscope and the microfluidic device. Saran wrap is placed on the microscope stage and the microfluidic device is placed on top of the saran wrap. The chamber is placed over the channel and the saran wrap is

pulled up and secured with a rubber band to complete the seal. Tubing connected to an external nitrogen tank enters the chamber and rapidly releases nitrogen gas. The gas displaces the oxygen creating a deoxygenated environment.

*Operation of Deoxygenation Chamber with Parallel Plate Rheometer:*

When the chamber is utilized with the parallel-plate rheometer, the split cover is used. First the cylindrical portion of the chamber is placed on the base of the rheometer. The blood sample is placed, then the parallel plate geometry of the rheometer is lowered. Finally, the split cover is secured around the shaft of the geometry. There will be tubing which enters the chamber to maintain the deoxygenation condition. When used with both the rheometer as well as the inverted microscope, the blood sample is initially deoxygenated in a separate facility before the use of the chamber. Once the sample is in the chamber, the deoxygenation of the sample is maintained through an influx of nitrogen introduced to the chamber. The nitrogen gas displaces the oxygen creating a deoxygenated environment. While some small leaks will likely occur due to the necessary split in the cover that allow the rheometer geometry to rotate freely, the positive pressure of the nitrogen used to deoxygenate the chamber will keep to a minimum the environmental oxygen entering the chamber through any small gaps.

*Operation of Microfluidic Rheometer:*
*Closed channels*
*Materials and Equipment:*

- Microchannels of closed channel configuration
- Reference fluid of known viscosity (distilled water is suggested)
- Blood sample
- Pipette
- Microscope
- Ruler

1. Place the glass slide containing the closed channel under a microscope.

2. Pipette 10ul of reference fluid and place at the opening of the closed channel.

3. Wait for the reference fluid to stop moving within the channel.

4. Measure the length of the distance the sample moved within the channel.

5. Repeat the measurements for each of the 4 remaining closed channels. These measurements will be used to calculate the capillary pressures for each of the varied channel widths using equation

6. Repeat this process with 10 μl of blood.

*Serpentine Channels*

*Materials and Equipment:*

- Serpentine microchannels
- Reference fluid of known viscosity identical to that used with closed channels
- Blood sample
- Pipette
- Timer
- Ruler or other means of measurement such as imaging software

1. Place 10 µl sample of blood at the inlet of the serpentine channel with the smallest width and immediately begin recording the time using the chosen timing method.

2. Note the position of the meniscus of the fluid sample and the corresponding times at various lengths of travel through the serpentine channel.

3. These measurements will be used in the calculation of viscosity and each represents a different shear rate. The number of points and location are determined by the user. The greatest shear rates occur immediately after the sample is placed and the lowest as the fluid travels towards the end of the channel.

4. Repeat the process using the reference fluid.

*Note: The value of the reference velocity multiplied by the length travelled in the channel should be roughly the same for all positions. It is recommended to take an average of these values and insert for the value* $vL_{calibration}$ *used to calculate the* $\frac{d^2}{S}$ *term of equation 15.*

*Calculation of Blood Sample Viscosity:*

The data obtained using the protocols for testing with the closed channels and serpentine channels is used for the calculation of the unknown blood sample viscosity using equations 14 and 15 as described in section 2.2.2.

The experimental procedures used to measure the lengths and times associated with the progression of the sample fluids through the microchannels are ultimately determined by the user and may take into account the number of desired data points as well as the range of shear rates at which the viscosity is to be calculated. An overview of the experimental procedure utilized for the testing in this research is presented here as a suggestion for a potential testing protocol. However, experimental setups are not limited to those described here.

*Comparison of Data:*

After fabrication of the device, a comparison of the device performance versus that of a conventional parallel-plate rheometer was made to determine the accuracy of the microfluidic rheometer. It was shown here that the data produced using the fabricated device indicated correlation with data acquired using the conventional rheometer as shown in Fig. 23.

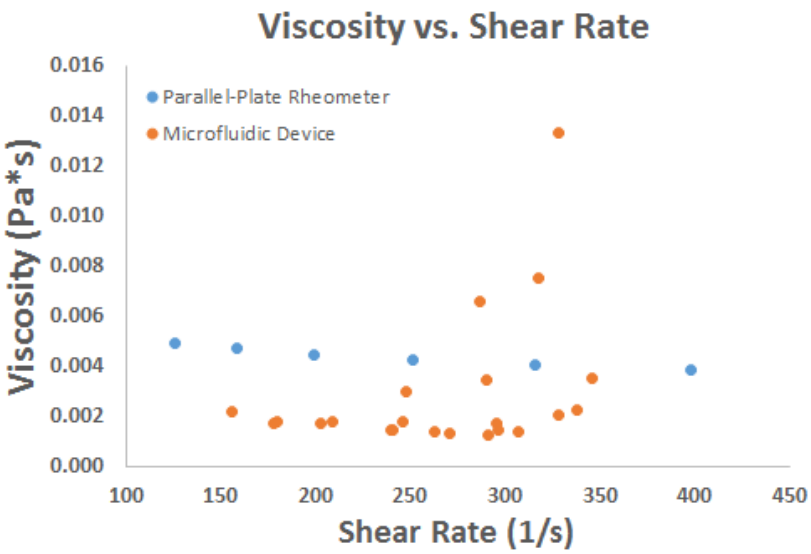


**Fig. 23.** Viscosity vs. shear rate for measurements made using the microfluidic device and a parallel-plate rheometer shown in orange and blue, respectively.

The outliers seen in the data correspond to the most narrow portion (200 µl in width) of the open serpentine channel. This may be due to the width of the channel being too narrow to allow for non-turbulent flow of the liquid sample. Future serpentine channel designs should incorporate widths between 200 µl and 350 µl to determine the minimum threshold of non-turbulent flow. The shear rates produced by the device range from ~150 $s^{-1}$ to 350 $s^{-1}$. Because blood is a non-Newtonian fluid that exhibits shear thinning, viscosity decreases as shear rate increases until a steady consistency is obtained. Within the human cardiovascular system, human blood viscosity remains approximately constant as shear rates increase above 300 $s^{-1}$ due to the fact that above this shear rate, blood plasma dominates the behavior of whole blood. As shear rates decrease below 300 $s^{-1}$, however, human blood viscosity increases exponentially as the interactions between red blood cells dominate the behavior of whole blood. Future work would involve increasing the total length of the serpentine channel to expand the shear rate range produced by the device. Due to an inverse relationship between shear rate and vessel diameter, the largest vessels in the body (e.g. aorta) exhibit low shear rates and the smallest vessels (e.g. capillaries) exhibit high shear rates. To obtain a shear rate range that is even more clinically meaningful, the minimum shear rate should be around 20 $s^{-1}$ to better mimic the shear rates experienced in the largest vessels of the body.

*Optional/Additional Equipment:*
*Digital camera:*

In this research, a digital camera was used to image the sample fluids in the device. We had limited time for fabrication of devices due to obstacles encountered during the design process and thus wanted to record testing to ensure a maximum availability of data for analysis. In this setup, we used a digital camera with a macro lens and manual focusing capability with the ability to record video.

The digital camera should be placed above the device at a height that allows for the channels to be clearly observed and in focus.

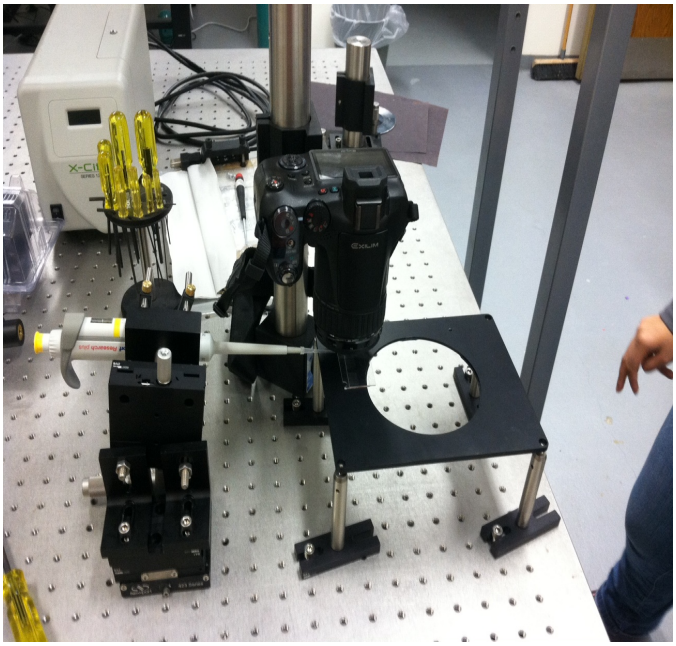

**Fig. 24.** Casio EXILIM Pro EX-F1 6.0 MP Digital Camera secured at proper height above device.

To focus the camera, the mode is set to "macro" and "auto focus" is turned off. When recording a video, the timer is set to 10 seconds to allow for sufficient time between beginning the recording and ejecting the sample from the pipette. Additionally, it is important to prevent too much light exposure as this may interfere with the quality of the video or image. It should be noted that the digital camera is highly sensitive to subject distance and light exposure and it may require several attempts to obtain a focused image. The sample is ejected and allowed to travel the entire length of the channel prior to stopping the video. Post-recording, video and images may be analyzed using image processing software. The known channel widths may be used to set the length scale of the obtained images and video, enabling the determination of the distances travelled by the sample fluids within the channels. Noting the time corresponding to particular frames, it is possible to determine the velocity of the fluid through the channels. In the experimental testing done during this research, Matlab was utilized for video analysis while Image J was used for the analysis of still images.

*Microscope*:

To observe the channels and fluid within the channels at a microscopic level, it is important that the device is properly placed on the stand of the microscope of choice. If a confocal microscope is used, the device should be placed on the stand and positioned using the x- and y-axis knobs such that the area of interest appears on the monitor.

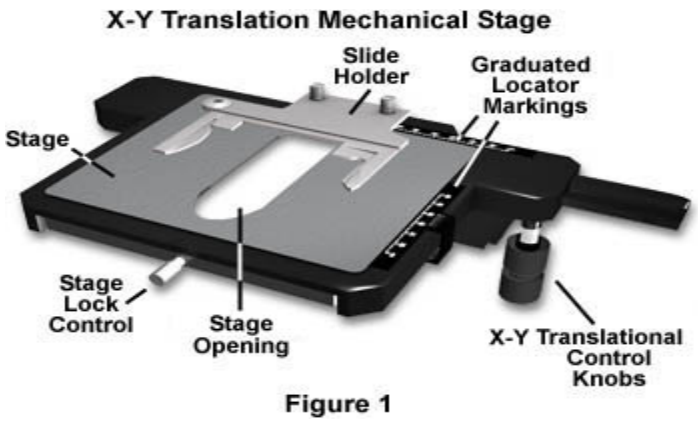


**Fig. 25.** X-Y translation mechanical stage of confocal microscope with X-Y translational control knobs. (image: http://micro.magnet.fsu.edu)

In this research, Image J was used in conjunction with a confocal microscope. Using this setup, images can be taken by leaving live view in the software and selecting "snap." To enter live view again, select "live." If a brightfield microscope is being used to observe the device, it is recommended that the lenses are switched to darkfield to provide greater contrast resulting in a more distinct visualization of the channels. It was found here that using the microscope only provided a limited field of view and that it was not possible to image the entirety of the channel at one time. Therefore, the microscope was most useful for viewing the fluid in the closed channels as this fluid does not move continuously, unlike fluid in the serpentine channels. The microscope, however, is well-suited for characterizing the qualities such as smoothness, continuity, etc. of fabricated microchannels.

*Pipette:*

Here, a pipette holder was assembled using Thor components and mounted on a Thor optical table. The setup incorporated micropositioners for accurate and stable placement of the pipette tip at the inlet of the microchannels during testing and filming.

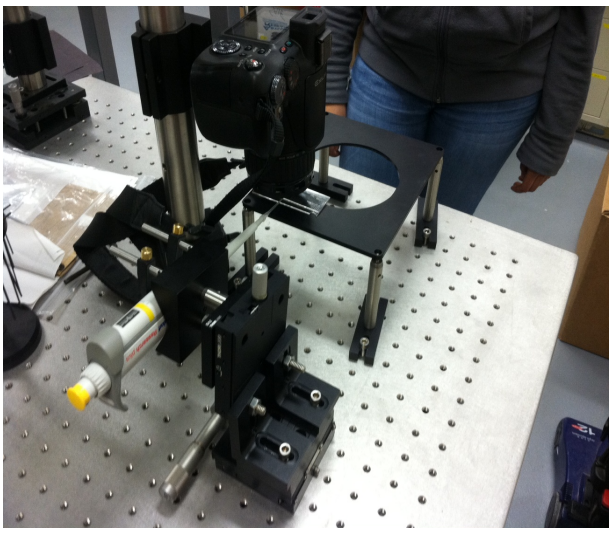

**Fig. 26.** Pipette holder in which pipette is properly placed.

The purpose of the pipette holder is to stabilize the pipette so that a wavering tip, which may distort image clarity, is not apparent in the video recordings. The pipette tip must be changed between samples to avoid cross-contamination which may alter results. Pipette tips should be properly disposed of in a biohazard sharps bin. To change the pipette tip and reload with sample in the shown setup, the pipette must be removed from the holder and replaced prior to the subsequent test. In addition to proper placement within the pipette holder, the pipette must also be properly placed at a sufficient height and distance from the channel inlet. Sufficient space between the pipette tip and channel inlet allows for the sample droplet to fall out of the tip immediately onto the inlet and drawn in instantaneously. It should be noted that not all of the sample liquid will be drawn into the channel allowing for excess fluid to run down the side of the device and seep into an adjacent channel causing contamination and rendering it potentially unusable. To prevent this from occurring with the serpentine channels, it is recommended that a small piece of tape be placed on both sides of the channel inlet to restrain the droplet from sliding along the device. It was also found that cutting channels apart after etching is another feasible alternative to prevent this issue.

## 3. Realistic Constraints

### *3.1 Client-stated constraints*

*Portability:* The client has indicated the importance of the device being portable. Ideally, the device should be operable outside of a hospital or laboratory setting, such as in the field. It is not necessary that the deoxygenation chamber be portable.

*Cost:* The device should be of low cost to manufacture.

*Simplicity:* The client has indicated that the device should be simple in both design and operation such that it is easy to construct and little training is required to use the device.

*Operability:* The device should be operable under both oxygenated and deoxygenated conditions. The client's current laboratory setup uses nitrogen as a means to maintain a deoxygenated environment when working with SCD RBCs. Thus, the materials utilized for the construction of both the rheometer and the deoxygenation chamber must be compatible with nitrogen.

### *3.2 General constraints*

*Environmental:* Experimental preparation is conducted within a sterilized cell hood to avoid contamination of blood samples from bacteria, random particles, etc. due to lab environment exposure. This careful precaution also serves to limit contact between blood samples and the general lab area thus protecting the lab itself and all lab personnel from exposure to bloodborne pathogens. The materials used for the manufacture of the device are disposed of according to University of Connecticut Environmental Health and Safety protocols. Proper handling procedures are followed for handling of chemical and biological agents and for any materials for which previous protocols did not exist the Division of Environmental Health and Safety at the University of Connecticut was consulted. All group members will receive Lab Safety and Chemical Waste Management training, Biosafety-General training, and Bloodborne Pathogens training through the Division of Environmental Health and Safety.

*Manufacturability:* The microfluidic devices used to conduct these studies were manufactured in-house when possible. The client provided laboratory space when possible while some procedures such as spin coating, photoresisting and etching were done in other laboratories within the University. For the construction of the deoxygenation chamber, the machine shop at the University was used and provided the necessary equipment for the construction of the entirety of the design. Group members received Machine Shop Safety training as required by the University of Connecticut prior to using the machine shop equipment. In terms of commercial production, if the viscometer design is to be fabricated on a large scale the microfluidics platform offers a cost effective means to do so [17]. Wet chemical methods similar to that used for the etching of the microchannels are used for manufacture of circuit boards and demonstrate the scalability of the process to a commercial level. Additionally, the deoxygenation chamber design is suited for scale up as it is composed of relatively few parts. The design of the PCB-constructed portions of the chamber could easily be made via injection molding, also adding to the ease of scale-up.

*Sustainability:* With proper care, the closed channels of the microfluidic devices involved in these studies are capable of being reused. Due to the nature of the design, the serpentine microchannels are not reusable. The device, however, is designed to be as compact as possible and to produce the minimum amount of waste while meeting the objectives of the project.

*Ethics:* The project held a limited budget. To conserve funds, parts were developed in-house whenever possible. While additional supplies were needed, thorough research was conducted prior to purchasing to assure utmost reliability, effectiveness and cost-efficiency. All funds used were solely for the purposes of this research. The client was kept updated on budget planning and consulted prior to any deviations from the planned use of funds. The potential applications of the device to healthcare in areas of limited income was kept in mind during the design process and the projected commercial cost of the final device was accordingly kept to a minimum.

*Health and Safety:* All group members will have undergone biological and chemical lab safety training prior to working in the lab. Microfluidic devices, rheometer components and anything else that comes in contact with the blood samples were be properly cleaned after every experiment.

*Political and Social:* To our knowledge, there are no political or social constraints on the project at this time.

## 4. Safety Issues

Individuals working with the device or manufacturing the device may potentially be exposed to hazardous chemicals or biological pathogens. Manufacturing of the device includes the use of chemicals that may be harmful to the health of personnel. The protocol for the photoresist masking of slides calls for the use of chemicals such as RD-6 developer and PR-4000A photoresist that may cause skin and eye irritation. With the fabrication process exposure to the RD-6 developer and PR-4000A photoresist is minimal. Through the use of personal protective equipment such gloves and goggles exposure to these harmful chemicals can

be minimized. Further, these chemicals are only used in a hood to further minimize exposure to personnel.

The wet chemical etching process used to create microchannels in glass slides requires the use of hazardous chemicals such as hydrofluoric acid and hydrochloric acid. Both of these reagents have the potential to cause serious injury or death. They should be handled with extreme caution within an appropriate fume hood only. Users should wear adequate personal protective equipment including laboratory coats, goggles, and two pairs of gloves. A hydrofluoric acid spill kit containing calcium gluconate gel should be immediately on hand in the case of environmental or human exposure. Individuals handling these chemicals should be familiar with the hazards and should have undergone the appropriate chemical safety trainings as required by their institutions. Upon completion of use of the reagents, they should be properly disposed of according to regulations.

When bonding the etched slides to a clean glass slide there is exposure to possible hazardous fumes from the glue. The optical glue is composed of methacrylate and UV resins. During application there are fumes released. These fumes are not toxic with minimal exposure but, long term exposure is harmful. It is important to work in a well ventilated facility when using the optical glue.

Individuals working with the completed device will come in contact with biological samples, and therefore are at risk of being exposed to possible pathogens. However, the user of the device is never directly in contact with the blood sample. The blood sample is transferred using a pipette from one closed container to the inlets of the device where the sample is drawn into the channel. In addition, the device requires only a droplet-sized sample to function, so a simple needle prick to the finger will suffice to obtain the blood sample. With such a small sample the exposure to blood is minimized, thus the risk of contracting any blood borne pathogens is reduced. While the risk of exposure is small, universal precautions should still be taken and the proper personal protective equipment utilized.

## 5. Impact of Engineering Solutions

WBV is recognized as an indicator of health in various disorders including cardiovascular disease and SCD [1][7]. Cardiovascular disease is a group of diseases that includes coronary heart disease, deep vein thrombosis, and peripheral arterial disease [8]. According to the World Health Organization, more people worldwide die annually from cardiovascular disease than from any other cause, a trend that is expected to continue in the future [8]. More than 80% of these deaths occur in low- and middle-income countries [8].

SCD is a blood disorder in which the body makes blood cells that become crescent shaped when deoxygenated as opposed to maintaining the biconcave shape of healthy cells. Individuals affected by the disease experience a range of symptoms such as chronic pain, necrosis, and stroke [3]. It is estimated that the disease occurs in >200,000 births per year in Africa and between 60,000 and 100,000 births per year elsewhere [2].

It has been shown that WBV is generally higher in patients with cardiovascular disease or SCD. It is clear that measurement of WBV is of clinical importance to better assess the health of patient. Our optimal design provides an effective solution in that it allows for quick, simple measurement of WBV using a small sample of blood. Additionally, the prevalence of diseases affecting blood viscosity in low- and middle-income countries that may not have widespread access to laboratory facilities makes desirable a device that can be utilized in the field. While

other research has been done on the measurement of WBV, the construction of such devices limits their applicability outside of a hospital or laboratory setting. The device by Kang, et al., allows the measurement of WBV at any desired shear rate, but utilizes a pumping mechanism that limits the simplicity, portability, and cost-effectiveness of the device [10]. Srivastava, et al., demonstrate a device that uses no external pump but that is limited to a low range of shear rates as determined by the viscosity of the tested sample [11][16]. Our design provides a novel engineering solution in that it facilitates the measurement of WBV over the range of shear rates present in the human body without the use of an external pumping mechanism. These qualities contribute to the applicability of the device as a point-of-care measurement for individuals with conditions affecting WBV that may not have regular access to medical care. The low cost, portability, simplicity, and practicality of our device have the potential to benefit clinics around the world, while the applications of the device can yield meaningful data that can positively impact public health by means of early diagnosis, prognosis, and disease prevention.

## 6. Life-Long Learning

At the conclusion of this project, team members have acquired a variety of skills that are relevant to industry and academia and are translatable to their future pursuits. First, the wet chemical glass etching process utilized to create the microfluidic devices is a valuable technique that has been introduced to us. This process involves a variety of materials such as PR-4000 photoresist and RD-6 developer to mask a glass slide prior to etching with a solution of hydrofluoric acid, hydrochloric acid and distilled water to form microfluidic channels. We also learned how to bond etched slides to plain glass using optical glue cured with UV light. In addition to these materials, we have been introduced to other devices and techniques that play a role in the etching process. We were familiarized with the operation of the equipment in the Chemistry Clean Room at the University of Connecticut including a spin coater, UV light source and mask aligner. We also underwent the appropriate trainings to handle chemicals as well as biological samples.

To create the mask applied when exposing UV light to specific regions of PR-4000 photoresist, the design software SolidWorks is utilized. This program is an important tool as it played a vital role in the development and success of our design. Furthermore, as a means for creating clear and effective schematics, SolidWorks greatly assisted in the the overall presentation of our final product. We have also learned how to use a rheometer to measure the viscosity and shear rates of our samples. The rheometer provided measurements to be used as our standard comparison. Associated with the rheometer are the data acquisition and analysis programs which are essential skills that have also been acquired. Moreover, the rheometer and associated computer software depend on the geometry used during testing. There exist a variety of geometries such as a flat surface geometry and cone-shaped surface geometry that we have been made aware of, although only the flat geometry was used in our studies.

**7. Budget**

| Item | Company | Total ($) | Balance ($) |
|---|---|---|---|
| PEO | Sigma Aldrich | 44.88 | 955.12 |
| Mask 1 | CAD/Art Services | 63.50 | 891.62 |
| HCL | Sigma Aldrich | 276.86 | 614.76 |
| HF | Sigma Aldrich | | |
| Glass slides | Sigma Aldrich | | |
| Acetone | Sigma Aldrich | | |
| Positive Photoresist | Futurrex, Inc. | 383.00 | 231.76 |
| Developer | Futurrex, Inc. | | |
| UV lamp | Summers Optical | 204.00 | 27.76 |
| Optical Glue | Summers Optical | 88.00 | -60.24 |
| PCB | MCMaster-Car | 38.94 | -99.18 |
| Mask 2 | CAD/Art Services | 38.50 | -137.68 |
| Deoxygenation Chamber | Machine Shop: UConn | 25.00 | -162.68 |
| Spill Kit | Calgonate Inc | 361.00 | -361.00 |
| Beakers | Chemistry building Stores | 41.28 | -41.28 |
| **Total** | | **1564.96** | **Over Budget by $564.96** |

**8. Team Member Contributions to the Project**

The work completed on this project was accomplished as a team in a manner that was fair and equal among the members. Throughout the course of the project, project-specific tasks were divided up and assigned to individual team members, either by the client or the team itself, or performed together as a team. All tasks were carried out effectively and efficiently by the members of the team. Specific contributions to the project by each team member will now be elaborated upon.

Catherine contributed to the project by attending training sessions regarding lab safety, rheometer testing and software analysis as well as microchannel fabrication by means of soft lithography. She also assisted in the completion of preliminary tests with the rheometer using ketchup as the fluid under observation. Catherine contributed to the theoretical development of the alternative designs involving electrowetting to create a microfluidic device. Once the optimal design was selected, she then worked with SolidWorks to assist in the creation of a 3-dimensional schematic of the preliminary device. Catherine also worked with Matlab to determine the range of shear rates that this device would be capable of producing with varying dimensions. Throughout the first semester, Catherine completed her assigned portions of the project reports and weekly meeting presentations and always made sure to complete her tasks on time and to the best of her ability.

Catherine continued to work diligently throughout the second semester. She took part in the purchasing of all new laboratory and safety materials for glass etching and was actively involved in the fabrication of microchannels using this new technique. Catherine also worked in the clean room to spincoat and develop many channels using photoresist and developer, respectively. Upon fabricating testable devices, Catherine dedicated several hours to learning how to properly focus and record a video of the sample fluids traveling through the channels. Subsequent to testing, she assisted in the completion of data analysis. Throughout the second semester, Catherine contributed by completing equal portions of the project and project reports.

Jessica contributed to the project by attending trainings including sessions on Lab Safety and Chemical Waste Management, microfluidic fabrication methods, blood sample preparation, and rheometer operation. With other teammates, she performed experiments with non-Newtonian fluids with a parallel-plate viscometer. Jessica contributed to the project through research on topics including rheology, SCD, cardiovascular disease, non-Newtonian fluids, cardiovascular shear rates, capillary flow, and microchannel surface modifications. She also researched previous work on similar devices done by others. Jessica contributed ideas to the final design of the microfluidic viscometer and designed the deoxygenation chamber. She also gained experience with Solidworks and modeled the deoxygenation chamber using this software. Jessica also contributed to the weekly presentations and in the writing of project reports.

Jessica contributed throughout the second semester of the project by working with her team members to fabricate and test the device prototype. Tasks included ordering supplies, spin coating and developing glass slides in the clean room, chemical etching of microchannels and bonding of channels with optical glue. Additionally, Jessica assisted in the testing of the fabricated device that involved filming and data analysis as well as comparative testing made with a parallel-plate rheometer.

During the first semester Divya contributed to the project by initially conducting preliminary research on the concepts of rheology, non-newtonian fluids, microfluidics and rheometer. She later than conducted a thorough review of papers by Srivastava and Burns which was used to developed our final device design. Divya learned the basics of solidworks to create the design of the device on solidworks and also produce the mask design required for the fabrication process. With her other teammates, Divya participated in learning and conducting the fabrication of the device using soft lithography. Towards the end of the fall semester, She attended the required machine shop training and conducted preliminary test of the device was fabricated using soft lithography.

During the spring semester Divya researched and learned the new fabrication method of wet chemical etching. She assisted with the purchasing of all the new materials and maintaining an accurate account of all the purchases. Her biggest contribution was working on the photolithography process of the wet chemical etching method which consist of spin coating, UV exposure, and developing. She worked on fine tuning the details of the method such as baking time and exposure time to produce the best result. She also assisted with chemical etching and bonding process. Towards the end of the semester she worked on testing device and analyzing the data using Image J and Matlab.

## 9. Conclusion

As previously stated, SCD impacts hundreds of thousands of people worldwide causing patients to experience a range of symptoms such as chronic pain, frequent infections, necrosis, organ damage, and stroke [3][4]. Furthermore, our client's current work involves research on SCD, which is known to affect WBV. While WBV is recognized as a point-of-care measurement in SCD and other diseases affecting WBV such as cardiovascular disease, current testing methods involve expensive equipment, are time consuming, require a large blood sample on the order of milliliters, and are not practical outside of a laboratory setting [5][6][7][9]. This may present a barrier to treatment, particularly in those areas in which individuals do not readily have access to healthcare and/or medical facilities.

The goal of this project was to design, fabricate, and assess the performance of a simple, inexpensive, portable device for the measure of WBV. Taking into account the client's particular interest in measurements of blood viscosity in SCD, the device was designed to function in both an oxygenated and deoxygenated environment. A microfluidic viscometer using capillary pressure to drive the flow of a reference fluid and a blood sample through an array of both open serpentine microchannels and closed microchannels was fabricated. The closed channels of the device allow the calculation of capillary pressure using the reference fluid while the serpentine channels are used to calculate parameters including fluid velocity that are used in the final calculation of the WBV of the sample. The device was tested and the results compared with a parallel-plate rheometer. The viscosity measurements made with the fabricated device indicated a correlation with those made using the conventional rheometer.

The proposed device overcomes limitations of currently available viscometers and allows for operation outside of a laboratory setting. It offers the novelty of portability, simplicity, and the measurement of WBV over a range of shear rates while meeting objectives specified by the client with a projected cost of ~$1.40 per device.

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**12. Appendix**

***12.1 Updated Specifications***

***Microfluidic Device***

To produce the microfluidic device we will be using a glass etching method.

Material Specifications:

*Physical:*

Glass

*Environmental:*

Storage Temperature: 25℃ - 55℃

Operating Temperature: 25℃ - 55℃

Operating Environment: Hospital or other clinical setting. Possible operation in the field.

***Deoxygenation Chamber***

Material Specifications:

*Physical:*

Polycarbonate (PCB)

*Environmental:*

Storage Temperature: Maximum temperature: 93℃

Operating Environment: Laboratory.

***12.2 Ethics***

Biomedical Engineering, as a discipline and profession, combines engineering techniques with biological sciences, medicine, and technology, all in an effort to enhance the quality of human life. Because the applications of Biomedical Engineering are designed to directly affect the general public, it is necessary to consider public health and wellbeing at all times. Biomedical Engineers must demonstrate ethical conduct in professional practice, research, patient care and training. According to the Biomedical Engineering Society Code of Ethics, to fulfill their professional engineering duties, Biomedical Engineers shall "use their knowledge, skills, and abilities to enhance the safety, health, and welfare of the public" as well as "consider the larger consequences of their work in regard to cost, availability, and delivery of health care." [22] When proposing a design for the microfluidic device, barriers to public health and safety such as limited access to healthcare facilities were taken into consideration. The device was, therefore, designed to be easily portable and simple to operate. Furthermore, the cost of the device was kept reasonably low at approximately $1.40 per device. To provide maximum comfort to the patient and reduce the possibility of biological exposure to the clinician operating the device, the size of the blood sample required for accurate test results has been minimized. Additionally, to keep record of material cost, an accurate budget was kept throughout the design

process. The BMES Code of Ethics also states that all Biomedical Engineers involved in research shall "comply fully with legal, ethical, institutional, governmental, and other applicable research guidelines, respecting the rights of and exercising the responsibilities to colleagues, human and animal subjects, and the scientific and general public" as well as "publish and/or present properly credited results of research accurately and clearly." [22] Throughout the course of this design project, all applicable research guidelines were abided by and the rights of others were respected. All external sources of funding, technical assistance and scientific information used in the completion of this project have been properly acknowledged and/or cited in this report and in all associated project presentations. Furthermore, the results and conclusions presented have not been altered or skewed in any way and represent our findings clearly and truthfully.

### *12.3 Additional Information*

See attached documents.